THE EFFECTS OF GLUCOSAMINE HYDROCHLORIDE ON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER [1]

M. T. M. RIZKI

Department of Biology, Reed College, Portland 2, Oregon

An unusual type of abnormality in *Drosophila* was encountered during a series of feeding experiments involving glucosamine hydrochloride. These abnormal pupae resembled the *cryptocephal* recessive lethal described by Hadorn and Gloor (1943). Since a high incidence of abnormal individuals was recovered after treatment, and none of the other sugars tested produced morphological abnormalities, a more detailed examination of the conditions responsible for this effect, as well as a histological examination of the abnormalities, was undertaken. This report summarizes the observations of the effects produced by feeding glucosamine hydrochloride to larvae of *Drosophila melanogaster*.

Materials and Methods

The tu^w mutant strain which develops melanotic tumors in the caudal fatbody (Wilson *et al.*, 1955; Rizki, 1957) and the *Ore-R* wild type strain have been used in the present study. The abnormal pupae were first noted in experiments in which larvae were removed from Cream of Wheat-Fleischmann's yeast medium and placed on paper strips moistened with a solution of glucosamine hydrochloride. This same method was therefore used in subsequent experiments, as well as merely adding the test substance to the medium on which larvae were feeding. Each group of larvae was collected during a two- to three-hour interval, and larval ages are counted from the time of oclusion from the egg. All experimental material was maintained at 23°–25° C. in an incubator.

In the series of starvation experiments, the timed larvae were removed from the food dishes and washed in a series of changes of a saturated solution of NaCl, a 2% solution of NaOCl, followed by at least six rinses of distilled water. The larvae were then placed on tissue paper strips (Kleenex brand) in sterile petri dishes with filter paper under the covers to prevent the escape of the larvae from the dishes. Distilled water was used to moisten the paper strips in the control dishes, and the test solutions were pipetted onto the paper strips in the experimental dishes. Conditions were standardized throughout the study by using four layers of paper, size 2.5 × 2.5 inches, with 2 ml. of test solution for each group of 15–20 larvae. In some experiments sterile cellulose pulp was used as the crawling site for the larvae in place of paper strips. In other experiments the sugar solutions were added to Cream of Wheat-Fleischmann's yeast medium. In these cases the larvae were washed as above and approximately the same area of the petri dish was covered with the food as the size of the paper strips used in the starvation ex-

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periments in order to maintain conditions as similar as possible throughout the study.

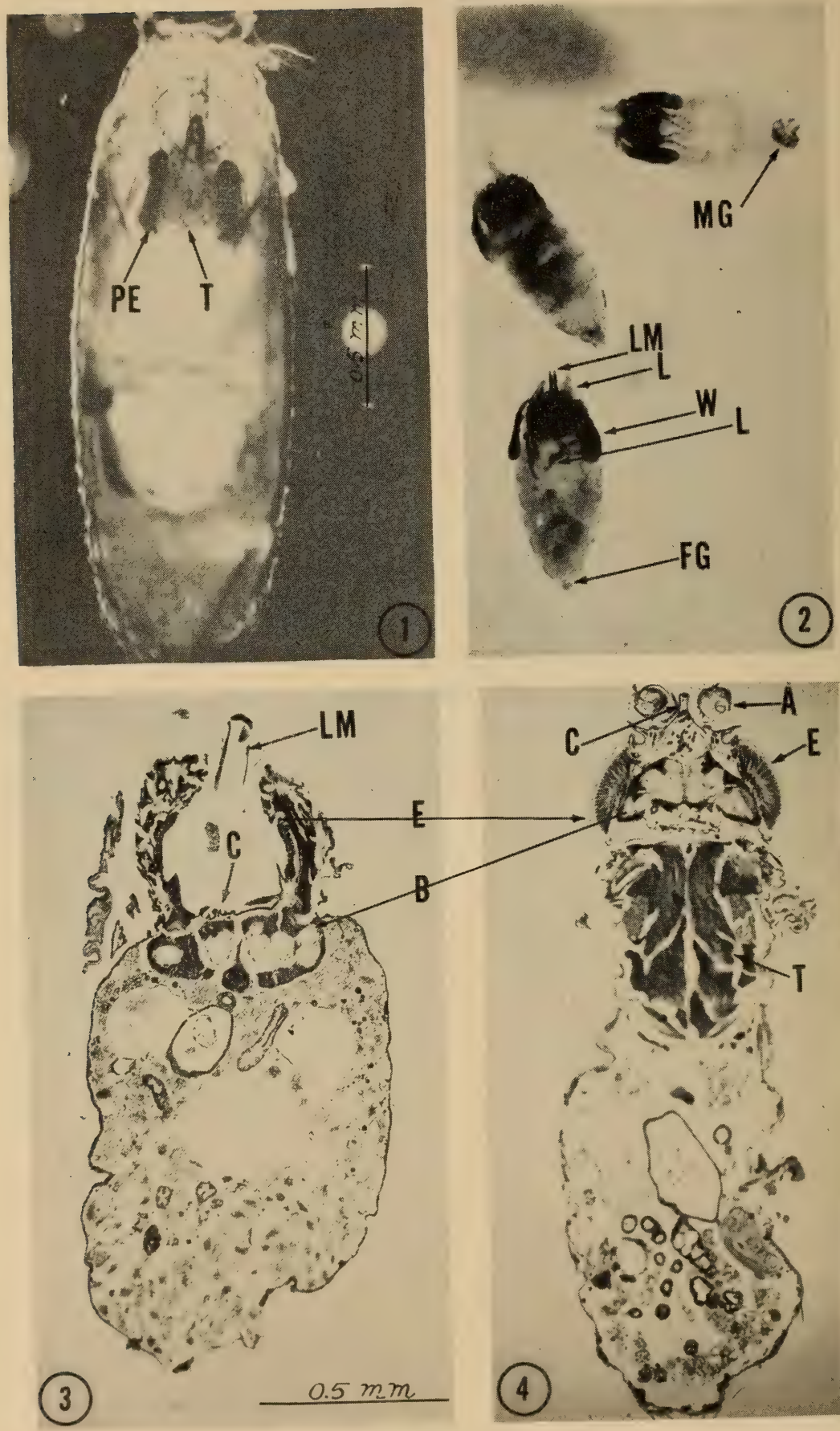
The abnormal pupae were dissected in Waddington solution. The histological material was prepared by fixing larvae and pupae in Carnoy solution, embedding paraffin, and staining the sectioned specimens with Azure B (Flax and Himes, 1952).

Results

The typical pattern of the abnormality induced by feeding glucosamine hydrochloride involved the disproportionate sizes and relative positions of the structures of the head and thorax. The most striking feature of the abnormal pupae was the reduced size of the eyes which nevertheless developed normal pigmentation (Fig. 1). Examination of pupae after removal of the puparium showed that the cephalic structures were contained in the thorax. The thoracic structures, including the legs and the wings, have developed, their distortion in position being a result of the presence of the head within the thorax. In some cases the legs appeared shorter than normal and were not fully stretched (Fig. 2). The larval mouthparts were still attached to the anterior end of the thorax, and many of the puparia were unusually elongated. The degree of contraction of the larval cuticle had been less in these cases. In some pupae the bristles were well formed but in others the development of these structures, particularly in the posterior region of the body, had been delayed. None of the abnormal pupae hatched, and sectioned material from these pupae was subsequently examined.

Stained preparation of the sectioned pupae revealed that eversion of the imaginal discs of the imaginal complex had occurred but these developing head structures did not emerge from the thorax. Figures 3 and 4 are frontal sections passing through the brain in an abnormal pupa and a normal specimen, respectively. In the former, the brain is located in the anterior region of the abdomen. Comparison of these sections indicates the degree of morphogenetic displacement which occurs in the brain mass. In the normal pupa, as the cerebral ganglion is pushed forward, the anteriolateral parts of the brain are displaced caudolatrad and the first optic stalks assume a lateral position to the brain. In the abnormal specimens the antennae and palpi appear normal with respect to their morphological development but they are found lodged in the area enclosed by the eyes. The carina is not properly formed. The gonads of the abnormal pupae are well developed and no apparent abnormality of the external genitalia would be detected. Eversion of the wings and legs has been complete. A parasagittal section of an abnormal pupa is presented in Figure 5 to illustrate the development of the thoracic muscles, tergites, and bristles. In this photograph, the compound eyes, although failing to emerge from the thorax, show well formed rhabdomeres and crystalline cones, and the setae are normal in appearance. Several pupae were found which had dwarf heads, and improper wing eversion was apparent in a few adults.

The first group of abnormal pupae was recovered during a survey of nutritional factors which were being tested for their effects in the mutant strain, tu^w. For these experiments larvae 65 hours of age were removed from food and placed on paper moistened with a 10% solution of each test substance. Larvae remained in these dishes until after pupation, and scoring was completed as the adults hatched. Under these conditions, glucosamine hydrochloride was the only chemical



FIGURES 1–4.

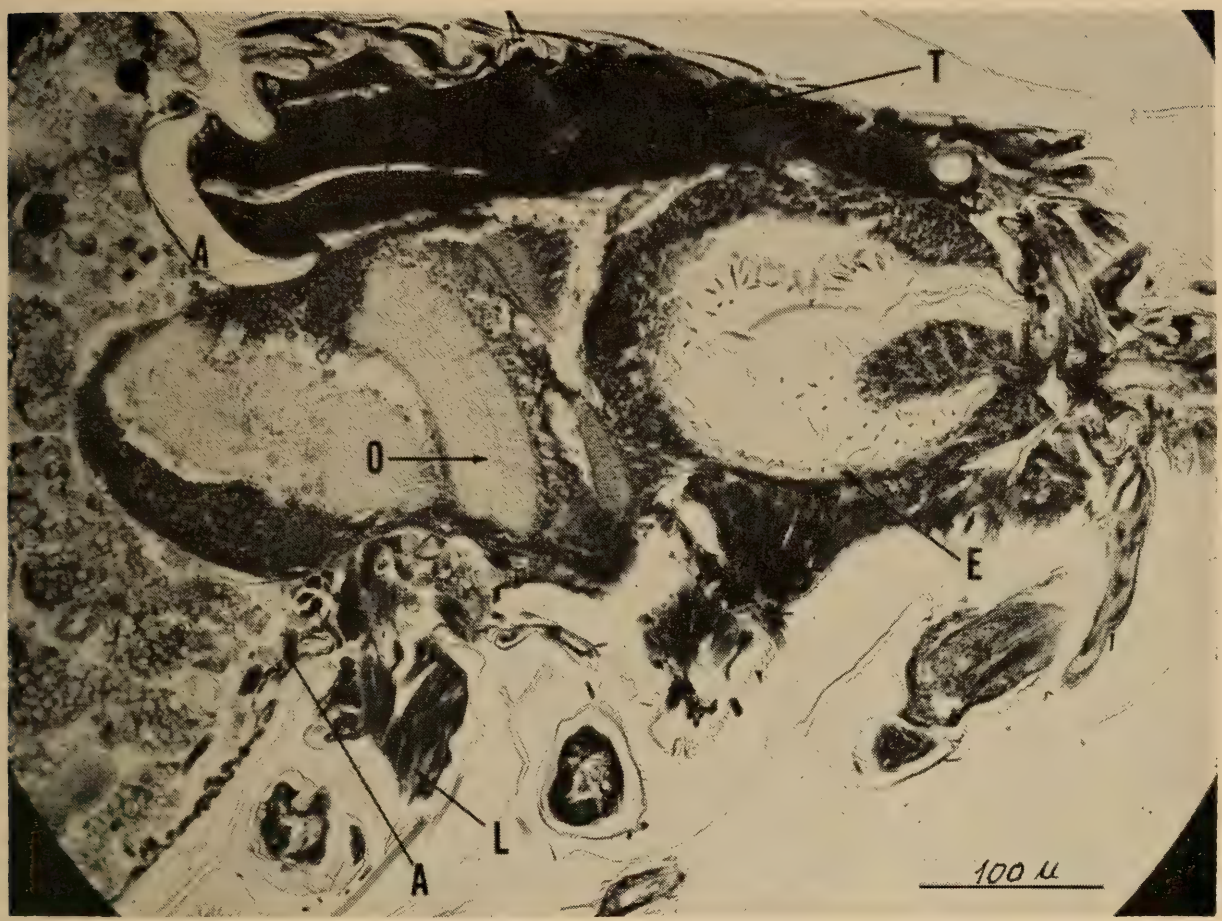


FIGURE 5. Parasagittal section of a glucosamine-induced phenocopy of *crc* in the *Ore-R* strain showing the detailed structures of the eye, E; optic lobe, O; longitudinal thoracic muscles, T; muscles of the appendages, L. A large portion of the brain is in the abdomen demarcated by A.

which induced any noticeable morphological changes. The list of substances tested included N-acetyl glucosamine, thiamine hydrochloride, fructose, glucose, lactose, starch, mannitol, salicin, casein hydrolysate, nucleic acid hydrolysate, glycogen, melibiose, trehalose, ribose, sorbose, inulin, sucrose, maltose and galactose. *Drosophila* larvae 65 hours of age were removed from food and placed on paper moistened with distilled water. These larvae completed their development and normal adults were obtained. Repetition of these conditions with glucosamine hydrochloride yielded the same type of abnormalities each time, and this method

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FIGURE 1. An atypical pupa within the puparium obtained after feeding glucosamine hydrochloride to third instar larvae of the *Ore-R* strain. The concave discoid pigmented eye, PE, is located in the thorax; the arrow, T, is pointing to the scutellar bristles of the thorax.

FIGURE 2. Atypical imagoes removed from the puparium (tu^w). Note the absence of the cephalic region while the thorax and abdomen with their associated structures are well formed. Female genitalia, FG; leg, L; male genitalia, MG; wing, W; the larval mouthparts, LM, still attached to the anterior region of the thorax. The legs are not fully stretched.

FIGURES 3 AND 4. Comparison of frontal sections of a glucosamine-induced abnormal pupa (tu^w) and a normal tu^w imago. In the abnormal specimen in Figure 3 the brain is located in the abdomen and the eye discs are flanking the inner lateral sides of the thorax forming a cavity. Larval mouthparts, LM; carina, C; antenna, A; eye, E; muscles of flight in the thorax, T.

was used to test 1%, 5%, 10%, and 20% solutions of glucosamine hydrochloride and N-acetyl glucosamine in succeeding experiments. In addition these same solutions were added to a thin layer of food covering approximately the same area of the dish as the size of the paper strip.

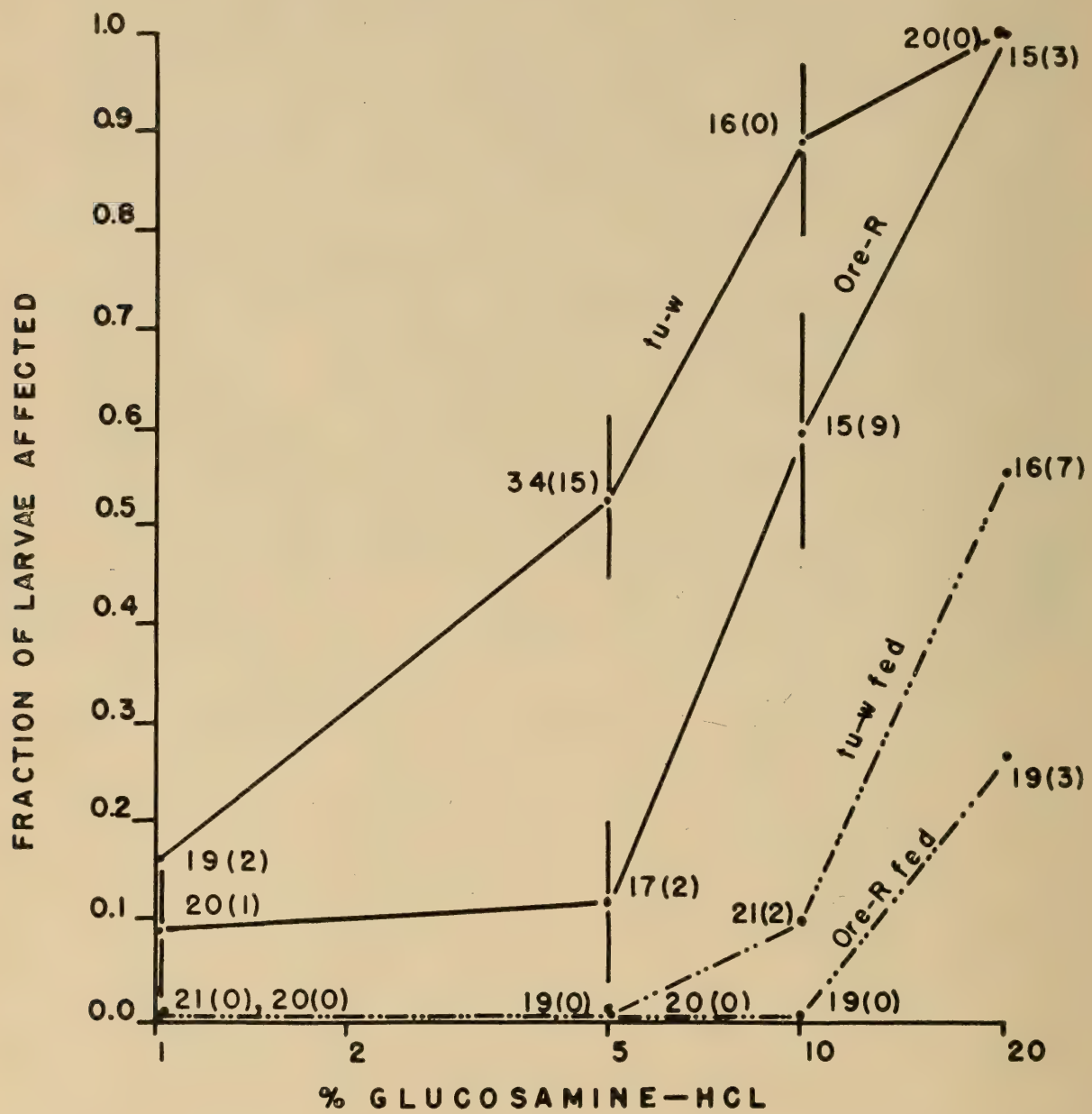

FIGURE 6. The yield of abnormal pupae and larvae in the tu^w and *Ore-R* strains obtained after feeding varying concentrations of glucosamine hydrochloride. The solid lines represent starvation conditions in the tu^w and *Ore-R* strains, and the vertical lines on these two curves indicate standard deviations. The results on the fed larvae are represented by dotted lines. All of the larvae used in this experiment (#756) came from one collection period. The fraction of larvae affected includes the total effect of feeding glucosamine hydrochloride, that is, abnormal pupae as well as larvae which died before pupation. At each point on the graph, the number preceding the parenthesis is the sample size which is followed by a number in parentheses giving the count of the pupae at this point with the *cryptocephal* phenocopy. For example, the higher per cent of phenocopies in the tu^w strain under starvation conditions was obtained with 5% glucosamine hydrochloride; no phenocopies were obtained in the tu^w strain with 10% and 20% glucosamine hydrochloride as the larval lethality approached 100%.

None of the tu^w larvae survived in the 20% solution of glucosamine under starvation conditions, and very few of the *Ore-R* larvae pupated. Many of the larvae crawled off the paper at this concentration, so the high mortality is presumably the combined effect of the glucosamine as well as desiccation. Behavior of this type was not noticed in the dishes with the lower concentrations of glucosamine. Combining the number of dead larvae and abnormal pupae obtained, the fraction of response to glucosamine hydrochloride can be graphically represented, thus indicating the difference between the two strains of flies. Figure 6 illustrates the results of one experiment on larvae taken from the same collection period. Direct comparison between larvae on food and those on paper strips cannot be made, but the difference most probably represents the dilution factor of the food since the same amount of solution was added to the food surface as that added to the paper in the dishes. The type of abnormalities induced was the same under conditions of feeding and starvation, as well as in the two strains of flies. The optimum concentration for the production of abnormal pupae was 5% for the tu^w strain and 10% for the *Ore-R* strain when larvae were removed from food at 65 hours of age, and these were the concentrations which were used in the experiments from which material was fixed and sectioned. In the present study, a total of 276 abnormal pupae was obtained from the tu^w strain and 99 from the *Ore-R* strain. Under optimum conditions of treatment the per cent of abnormal pupae in both strains was as high as 60%.

Samples of glucosamine hydrochloride obtained from three sources were used (Nutritional Biochemicals Corp.; California Corporation for Biochemical Research; Krishell Laboratories) and all produced the same type of abnormalities. The dosage response decreased with the age of the solution of glucosamine hydrochloride, so only freshly prepared solutions were used.

Discussion

The typical pattern of abnormality obtained after feeding glucosamine hydrochloride to *Drosophila* larvae is the same as that reported by Hadorn and Gloor (1943) for the recessive lethal which they termed *cryptocephal, crc*. This name appropriately described the characteristic feature of the glucosamine-induced abnormal pupae with the head structures contained in the thorax. Histological examination of the abnormal specimens has shown that the cephalic complex has undergone development and eversion, and the principal morphological difference appears to be the displacement of structures resulting from the lack of emergence of the head. A similar developmental upset was obtained in two stocks, the wild type *Ore-R* and the tu^w mutant strain, although a higher percentage of abnormalities was induced in the latter strain under the same conditions of treatment. Comparing pupation time in the two stocks, there is an average delay of at least twelve hours in the tu^w strain. Therefore the difference in response to glucosamine may reflect a difference in developmental stage since the larvae from both stocks were of the same age at the time of treatment. A survey of the effects produced by glucosamine hydrochloride when offered at different ages throughout larval life in both stocks will have to be undertaken before this question of difference of response can be answered.

Gloor (1944) reported lack of emergence of the head in some of the pupae of a wild stock of *D. melanogaster* by using temperature shocks during the prepupal

period shortly before the normal time of emergence of the head. The use of glucosamine hydrochloride as a phenocopic agent may provide a useful tool in studying the physiological mechanism of emergence of the head in the developing pupa. The biological role of the aminosugars has been recently reviewed by Kent and Whitehouse in their monograph (1955). Glucosamine and N-acetylglucosamine are components of the polysaccharide polymer, chitin. Complexes of chitin and protein are important constituents of insect cuticle. The incomplete contraction of the cuticle, apparent in some *Drosophila* specimens after feeding glucosamine hydrochloride, may suggest this system as a point of interference. The reported inhibition of anaerobic glycolysis and O_2 uptake by glucosamine in leucocytes (Alonso, 1959) also suggests further examination for such effects in *Drosophila* larvae and pupae. The ease with which abnormal pupae were obtained under starvation conditions was utilized to obtain as large a number of specimens as possible for the present survey. However, this restriction of starvation may not be desirable for an analysis of the mechanisms underlying the formation of the abnormalities since the histological condition of the abnormal specimens obtained in starvation experiments was inferior to that in the fed experiments.

Summary

Abnormal pupae were obtained after feeding glucosamine hydrochloride to larvae from two stocks of *Drosophila melanogaster,* the *Ore-R* wild type strain and the mutant melanotic strain, *tu*^{w}. The characteristic feature of the abnormality was the position of the head within the thorax, thus suggesting the use of glucosamine hydrochloride as a phenocopic agent to produce individuals resembling the recessive lethal *cryptocephal.*

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